

Agronomic and Microbial Responses to Long-term Straw Return in Purple Paddy Soil

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Soil degradation threatened the fertility of purple paddy soil. A continuous field experiment with 12 years was conducted to evaluate the impact of long-term rice straw return on agronomic and microbial aspects of soil fertility and health. Compared with NPK fertilization alone (NPK), combined application of NPK fertilization together with straw return (NPKS) had higher soil organic carbon content and other tested parameters of soil fertility, resulting in an increase of 6.9% for grain yield and 10.5-16.2% for nutrient uptake, respectively. Concurrently, straw return flourished the amounts of fungi and especially actinomycetes and bacteria, and also boomed anaerobic bacteria such anaerobic cellulolytic bacteria, anaerobic fermentative bacteria, hydrogen-producing acetogen, methanogenic bacteria and denitrifying bacteria, but remarkably depressed the growth of anaerobic nitrogen-fixing bacteria. Both NPK and NPKS treatments increased activities of soil enzymes including invertase, urease, neutral phosphatase and catalase. Intensity of methanogenesis in soil with NPKS treatment was increased by 34.7%, whereas intensity of anaerobic nitrogen fixation was decreased by 37.2% when compared with NPK treatment. We concluded that straw return, as a simple and effective agronomic practice, should be recommended to sustain soil health and crop productivity in purple paddy soil.

Key words: Straw return; Purple soil; Soil fertility; soil microbes.

Rice (*Oryza sativa* L.) is one of the major staples, feeding more than half of the global population. To feed increasing world population on reducing croplands, an increase in rice production per unit area is direly needed¹. Preservation and improvement in soil fertility is undoubtedly a fundamental basis for increasing soil productivity, especially in developing countries². The soil fertility depends on lots of physical, chemical and biological soil properties. Soil microbes, the unseen majority of organisms in soil, play key roles in ecosystems and influence a

large number of important ecosystem processes, including nutrient acquisition, carbon (C) cycle, nitrogen (N) cycle, soil formation, genetic diversity, plant productivity, global warming and so on³. Changes in soil chemical and physical conditions due to land management such as varied fertilization influence microbial activity and population structure. Furthermore, microbiological and biochemical soil properties such as microbiological community structures and their biological activities have been seen as sensitive indicators and early predictors of changing soil quality or soil health^{4,5}.

A case study from organic farming illuminated that microbial quantity (biomass), diversity and soil enzyme activities were the key factors that improve soil fertility and maintain crop

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yields⁶. According to the results based on long-term experiments^{7, 8}, combined application of chemical fertilizers and organic amendments in traditional intensive agricultural system generally resulted in higher microbial quantity, soil biological activity and soil fertility than that application of chemical fertilizers alone.

Crop straw return (or incorporation) is a historically agricultural practice in China⁹ and other nations^{7, 8}. Straw return to soil can directly increase the organic C input and therefore sequester C in cropland soil and has great potential to mitigate greenhouse gas emission¹⁰. Furthermore, straw return shows many benefits in agro-ecosystem, including improving soil physical properties, increasing the accumulation of soil microbial biomass, strengthening the activity of most enzymes, changing soil humus components, accumulating available nutrients in soil, influencing crop growth and thereafter promoting crop yields^{7, 8, 9}. As a case in southern China, based on 94 field trials with one crop season, combined application of NPK fertilizer with straw return increased soil fertility index and rice yield by 6.8% and 4.4% respectively, when comparing with that of NPK fertilizer alone¹¹.

Purple soil (Purpli-Udic Cambisol) is one of the most fertile soils in China and is the most widespread soil type (1.1×10^8 ha) in the Sichuan Basin. However, degradation of purple soil, including impoverishment of soil organic matter and mineral nutrient in top soil and decline in soil enzyme activity due to soil erosion and overuse, is becoming a serious problem hindering the improvement of soil fertility and productivity¹². Rice cultivation is one of the major land uses there. As above mentioned, it is reasonable to expect that rice straw return together with application of chemical NPK fertilizer would increase soil fertility and thereafter rice yield in purple paddy soil. Up to now, however, little is known about the impact of long-term rice straw return in purple paddy soil on sustainable fertility and soil health especially in microbial aspect. The objective of this study therefore was to investigate the changes of soil fertility, rice yield, nutrient uptake, and specifically soil biological activity in response to long-term application of chemical fertilizers alone or in combination with rice straw return in soil using a long-term field experiment with rice.

MATERIALS AND METHODS

Experimental station

This study was conducted at one of the National Monitoring Stations of Soil Fertility and Fertilizer Efficiency, which is located on the campus of Southwest University, Chongqing, China (30°26'22" N, 106°26'22" E). This station locates in east Sichuan Basin with annual mean temperature of 18.3°C and annual mean precipitation of 1115 mm. This station is a typical neutral Purpli-Udic Cambosols, which accounts for 40% of all purple soil in Sichuan Basin. In 1991, the initial soil properties were measured and given as following: pH, 7.7 (water:soil ratio of 2:1); organic C content (SOC), 13.9 g/kg; soil Total N (TN), 1.52 g/kg; alkali-hydrolyzable nitrogen (available N, A-N), 83.1 mg/kg; Soil total P (TP), 0.53 g/kg; 0.5 mol/L NaHCO₃-extractable P (available P, A-P), 4.3 mg/kg; soil total K (TK), 21.1 g/kg; exchangeable K (available K, A-K), 88 mg/kg. The land was cropped with a traditional rice-wheat rotation between 1991 and 2002.

Experimental design

The field experiment consisted of 10×12 m plots arranged in a complete randomized design with three treatments: CK (no fertilizer application, as control); NPK (applying NPK fertilizers alone); and NPKS (NPK fertilizers together with rice straw return to the wheat crop). The rate of NPK fertilizer used for each crop during the experiments were 150, 32.7 and 62.3 kg/ha for both rice and wheat during 1991-1996, while were 150, 26.2 and 49.8 kg/ha for rice during 1997-2002 and were 135, 26.2 and 49.8 kg/ha for wheat during 1996-2002, respectively. The fertilization strategy was to apply 60% N and 100% PK prior to planting, and 40% N at the 3-4 leaf stage for wheat and at 2-3 weeks after rice transplanting. The rice straw with a rate of 7500 kg/ha was applied annually prior to wheat planting. Each plot was divided into four subplots as pseudoreplication.

Sample collection

Straw and grain were sampled from each subplot at physiological maturity of rice and wheat during 1991 to 2002. The shoot samples were oven dried and then ground for chemical analysis. After rice harvest in each year, a stainless steel auger was used to collect soil samples from plow layer (0-20 cm). The soil samples were air-dried and ground for analyses of soil fertility parameters (pH,

SOC, TN, TP, TK, A-N, A-P and A-K) with routine methods. During rice cropping season in 2002, fresh soil was collected at jointing, flowering and mature stage to incubate for measuring soil microbial population, soil enzyme activity and soil biochemical intensity.

Compositions and preparation of media and incubation of soil microbes

The aerobic bacteria, fungi and actinomycetes were incubated using spread plate method. And the anaerobic microbes were cultured with the method of Hungate anaerobic technique. The details of compositions and preparation of the medium, the procedure of incubation, and counting and calculating the populations of soil microbes were given in previous study¹³. After colonies were grown, colony forming units (CFU) were counted and the numbers of soil microbes in dry soil (DS) sample were calculated with a unit of CFU/g DS.

Determination of enzymatic activities and biochemical intensity in purple paddy soil

Determination of activities of soil enzymes including invertase, urease, neutral phosphatase and catalase were conducted following the method in textbook of microbial research¹⁴. The units of invertase, urease, neutral phosphatase and catalase were expressed as 0.1 mol Na₂S₂O₃ ml/d/g DS, NH₄-N μg/d/g DS, Phenol μg/d/g DS, and 0.1 mol KMnO₄ ml/30 min/g DS, respectively.

Determination of intensities of soil biochemical processes including inspiration¹⁵, methanogenesis¹⁵, denitrification¹⁶, anaerobic nitrogen fixation¹⁵ and sulfate reduction¹⁷ were conducted following their referred methods. The units of inspiration, methanogenesis, denitrification, anaerobic nitrogen fixation and

sulfate reduction were expressed as 10⁻⁶ mol CO₂/d/g DS, 10⁻⁶ mol CH₄/d/g DS, reduced NO₃⁻/total NO₃⁻, 10⁻⁷ mol C₂H₂/d/g DS, S²⁻ μg/d/g DS, respectively.

Data analysis

Means of each treatment are shown in the Tables and Figures. The data were subjected to a separate analysis of variance (ANOVA) for each parameter, and the least significant difference (LSD) at p<0.05 level was used to determine differences between treatment means. SAS software (SAS 8.0, USA) was used for all analyses.

RESULTS

Soil fertility as affected by long-term straw return

During the 12-year cropping, SOC, TN, TK was obviously declined in no fertilizer treatment (CK), while other agronomic parameters of soil fertility almost kept same with that in initial year (Table 1). Compared with CK, fertilization with NPK fertilizer alone (NPK) or together with straw return (NPKS) resulted in significant improvement in most of soil chemical parameters, with except of soil pH which was substantially decreased. NPKS treatment showed a further increasing trend in SOC and other soil nutrients when compared with NPK treatment (Table 1).

Similarly with soil nutrient status, CK treatment maintained a low rice grain yield with an average of 3751 kg/ha; while NPK fertilization alone or together with straw return increased substantially grain yield. The average yield of NPKS treatment was 6869 kg/ha, which was 6.9% higher than that of NPK treatment (Fig. 1). NPK fertilization also enhanced the macronutrient uptake by rice. Compared with NPK treatment, uptake of N, P and K by rice with NPKS treatment

Table 1. Soil properties in purple paddy soil as affected by varied long-term fertilization. The means across 12 years (1991-2002) were shown. The means with different low-case letters in same column indicate significant difference at P<0.05 level

Treatment	pH	SOC	TN	TP	TK	A-N	A-P	A-K
CK	7.8a	12.5c	1.19c	0.56b	19.9a	79.7b	3.7b	77.1b
NPK	7.2b	15.0b	1.40b	0.71a	20.2a	89.4a	16.8a	82.2ab
NPKS	7.0b	16.3a	1.55a	0.79a	20.7a	95.8a	18.3a	90.3a

Note: SOC, soil organic carbon; TN, total soil nitrogen; TP, total soil phosphorus; TK, total soil potassium; A-N, available nitrogen in soil; A-P, available phosphorus in soil; A-K, available potassium in soil. Unit of SOC, TN, TP and TK is g/kg, while unit of A-N, A-P and A-K is mg/kg, respectively.

Table 2. Enzyme activities of purple paddy soil as affected by varied long-term fertilization. The means with different low-case letters in same column indicate significant difference at $P < 0.05$ level

Treatment	Invertase(0.1 mol $\text{Na}_2\text{S}_2\text{O}_3$ ml/d/g DS)	Urease ($\text{NH}_4\text{-N}$ $\mu\text{g/d/g DS}$)	Neutral phosphotase (Phenol $\mu\text{g/d/g DS}$)	Catalase (0.1 mol KMnO_4 ml/30 min/g DS)
CK	5.8c	178.7b	89.1b	6.6c
NPK	9.0a	219.8a	153.4a	7.1b
NPKS	8.4b	222.1a	148.4a	7.3a

Table 3. Biochemical activity of purple paddy soil as affected by varied long-term fertilization. The means with different low-case letters in same column indicate significant difference at $P < 0.05$ level

Treatment	Methanogenesis (10^{-6} mol CH_4 /d/g DS)	Denitrification (reduced NO_3^- /total NO_3^-)	Anaerobic nitrogen fixation (10^{-7} mol C_2H_2 /d/g DS)	Sulfate reduction (S^{2-} $\mu\text{g/d/g DS}$)
CK	3.5c	52.3b	2.5a	6.7c
NPK	5.2b	54.9a	1.8b	7.7b
NPKS	7.0a	54.8a	1.1c	8.2a

was increased by 11.4%, 16.2% and 10.5%, respectively (Fig. 2).

Populations of soil microbes in response to long-term straw return

After 12-year of varied fertilizations, populations of bacteria, actinomycetes and fungi had been greatly changed, although bacteria were still the predominant microbes among treatments (Fig. 3). Compared with CK, fertilization prospered reproduction of fungi and especially actinomycetes

and bacteria. NPKS treatment resulted in highest amounts of all three major soil microbes, which was 19.4%, 62.7% and 143.7% higher than that of NPK treatment for fungi, bacteria and actinomycetes, respectively (Fig. 3). Purple paddy soil also created anaerobic environment for growth of anaerobic bacteria (Fig. 4). Compared with CK, fertilization with NPK fertilizer increased the amounts of anaerobic cellulolytic bacteria (ACB), anaerobic fermentative bacteria (AFB), hydrogen-

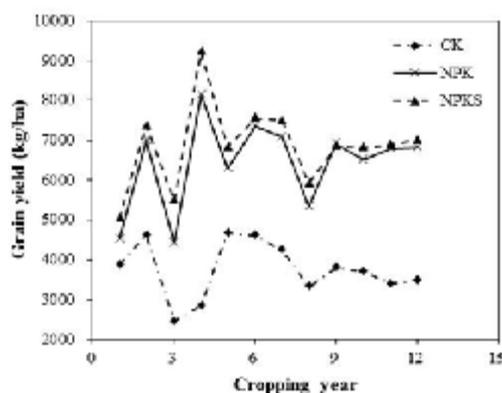


Fig. 1. Grain yield of rice grown in purple paddy soil as affected by varied long-term fertilization. CK, no fertilization (control); NPK, application of chemical NPK fertilizer; NPKS, combined application of chemical NPK fertilizer with rice straw return

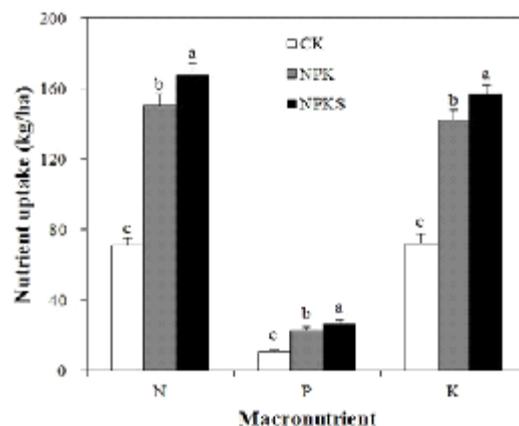


Fig. 2. Nutrient uptake of rice grown in purple paddy soil as affected by varied long-term fertilization. The means across 12 years (1991-2002) were shown. The means with different low-case letters in same set indicate significant difference at $P < 0.05$ level

producing acetogen (HPA), methanogenic bacteria (MB) and Denitrifying Bacteria (DB), all with highest values under NPKS treatment. Conversely, fertilization with NPK alone or especially with straw return decreased the amounts of anaerobic nitrogen-fixing bacteria (ANFB) by 14.9% and 52.3%, respectively. While, NPK fertilization alone has no effect on sulphate-reducing bacteria (SRB), but significantly increased with straw return (Fig. 4).

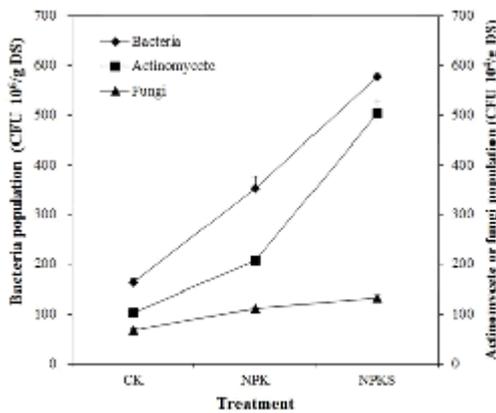


Fig. 3. Populations of major soil microbes in purple paddy soil after 12 years of varied fertilization. The means of three sampling stages during rice cropping season in 2002 were shown.

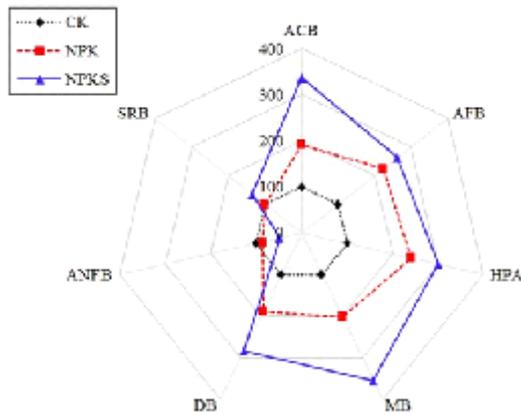
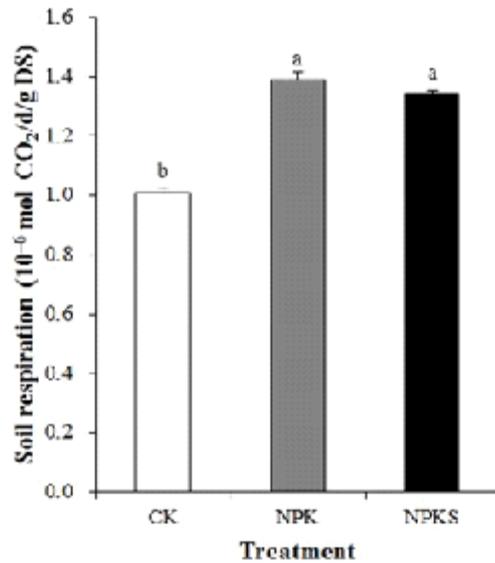


Fig. 4. Relative populations (%) of anaerobic microbe related with C, N and S cycle in purple paddy soil as affected by varied long-term fertilization. The values were expressed in percent of a given microbe in fertilized treatments accounted for that in control (CK). ACB, anaerobic cellulolytic bacteria; AFB, anaerobic fermentative bacteria; HPA, hydrogen-producing acetogen; MB, methanogenic bacteria; DB, Denitrifying Bacteria; ANFB, anaerobic nitrogen-fixing bacteria; SRB, sulphate-reducing bacteria.



The means of three sampling stages during rice cropping season in 2002 were shown. The means with different low-case letters in same set indicate significant difference at P<0.05 level.

Soil enzyme activity and soil biochemical intensity

After 12-year of varied managements, no fertilization resulted in lowest activities of all tested soil enzymes among the three treatments in 2002 (Table 2). Both NPK and NPK treatments substantially increased these enzyme activities, but there was no obvious difference between them. Compared with CK, fertilization resulted in significantly higher intensity of soil respiration with highest value under NPK treatment during rice cropping season in 2002 (Fig. 5). No fertilization resulted in lowest intensity of methanogenesis, denitrification and sulfate reduction, but resulted in highest intensity of anaerobic nitrogen fixation among the three treatments (Table 3). Compared with NPK treatment, straw return together with NPK fertilizer had similar intensity of denitrification, higher intensity of methanogenesis and sulfate reduction, but significantly lower intensity of anaerobic nitrogen fixation (Table 3).

DISCUSSION

In sustainable aspect of agronomic productivity, combined application of NPK fertilizer and straw return resulted in higher rice grain yield

than that of NPK fertilizer alone in purple paddy soil in 11 of 12 rice cropping seasons. This finding was consistent with past studies^{7, 11}. The reason was partly due to that chemical fertility of purple paddy soil was significantly improved under NPKS treatment when compared with CK or NPK treatments (Table 1). As a consequence of yield increase, NPK uptake by rice plant was further increased with NPKS treatment than that with NPK treatment (Fig. 2). This indicated an increase in nutrient use efficiency and a decline in nutrient loss to either waters or atmosphere, both of which are of importance for sustainable agriculture and environmental protection¹⁸.

The change of soil environment especially pH, SOC and soil TN would alter the population structure of major soil microbes. Compared with NPK treatment, NPKS treatment had higher amounts of fungi and especially actinomycetes and bacteria in purple paddy soil. This was well agreed with previous studies^{20, 21}. The possible reason would be that straw return together with N application resulted in increased SOC and soil TN, and nearly neutral pH value (Table 1), all of which were suitable for reproduction of bacteria and actinomycetes⁵. In anaerobic condition of paddy soil, combined application of NPK fertilizer and straw return increased amounts of straw-decomposition related bacteria (ACB, AFB and HPA). This indicated that flash organic matter by straw return to paddy field together with sufficient soil available N simulated reproduction of these bacteria. Conversely, straw return significantly reduced the amount of ANFB (Fig. 4), which indicated the increasing status of inherent soil N (Table 1).

Fertilization with NPK fertilizer alone or together with straw return increased tested enzyme activities, although the later had no obvious improvement than the former (Table 2). These soil enzymes play key role in cycles of C (invertase), N (urease), P (neutral phosphatase), and in microbial activity (catalase). The increase in these enzyme activities indicated a healthier soil under NPK or NPKS treatments than that without fertilization²².

Soil respiration was significantly enhanced by NPK fertilization alone or together with straw return (Fig. 5). This indicated an increase in overall activity of soil microbes in purple paddy soil with NPK fertilization alone or together with

straw return, which was consistent with previous study²³. The intensity of methanogenesis with NPKS treatments was significantly higher than CK or even NPK treatment (Table 3), which was well consistent with their quantities (Fig. 4). Such data was well matched with statistic data of field measurement that rice straw return stimulated CH₄ emission strongly²⁴. But taken the data of SOC, soil respiration, CH₄-related microbes and methanogenesis intensity together, straw return was still one of the most sustainable and economical carbon sequestration methods¹⁰. Compared with NPK fertilization alone, straw return together with NPK fertilization increased the amounts of SRB (Fig. 4) and intensity of sulfate reduction (Table 3), which was agreed with previous studies²⁵. The reason was mostly due to that decomposition of rice straw in paddy soil served as an important source of organic matter for SRP growth in the anaerobic process. Intensity of anaerobic nitrogen fixation was greatly decreased by straw return (Table 3), which was consistent with the declined amounts of anaerobic nitrogen fixing bacteria (Fig. 4), probably due to that the increasing status of inherent soil N depressed their population and activities.

CONCLUSION

Using a long-term field experiment in purple soil with rice-based cropping system, we revealed that NPK fertilization improved simultaneously the soil fertility, productivity and soil health, in the context of SOC and nutrient reservation, crop yield, nutrient uptake, populations of soil microbe and their biological activities. Furthermore, straw return together with NPK fertilization showed greater benefits in two interdependent aspects when compared with NPK fertilization alone. One was the agro-ecosystem sustainability in scopes of superior productivity, higher nutrient use efficiency, greater potential of C sequestration and greenhouse gas mitigation; another one was the biological scope of soil health where major soil microbes are flourishing and playing their roles properly. Therefore, straw return, as a simple and effective agronomic practice, was recommended to sustain soil health and crop productivity in purple paddy soil and probably in other similar soils.

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